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REMARKS

Claims 20-26 and 28-46 are pending in this application.

Applicants wish to thank Examiner Ramirez and Examiner Prouty for the helpful and courteous discussion with their undersigned Representative on April 7, 2004. Further, Applicants wish to thank Examiner Prouty for the suggestion to amend the claims to define the promoter sequence as a "DNA sequence situated at about position -35 from the transcription start site of the glutamic acid synthesizing gene" or a "DNA sequence situated at about position -10 from the transcription start site of the glutamic acid synthesizing gene" to clearly convey the scope of the claimed invention in terms that would be readily appreciated by the skilled artisan. During the discussion the remaining grounds of objection/rejection were discussed. The content of this discussion is reflected in the amendments and remarks herein. Reconsideration of the outstanding objections/rejections is requested.

The rejections of Claims 20-37 under 35 U.S.C. §112, first paragraph (written description and enablement), are obviated by amendment.

The present invention provides a method of constructing a mutant capable of suitably enriching or controlling the expression of an intended gene without using a plasmid and also capable of producing amino acids in a high yield, by the recombination or mutation (page 3, lines 7-10). To this end, the present method is accomplished by variously altering the promoter of amino acid-biosynthesizing gene on a chromosome to control the amount of the expression of the intended gene. Particularly, the present method is accomplished by introducing a specified mutation into -35 domain or -10 domain which is a specific domain of the promoter (page 3, line 25 to page 4, line 2).

In making the written description rejection, the Examiner concedes that the specification adequately discloses the production of coryneform bacteria having mutant promoters for the coryneform bacteria glutamate dehydrogenase, citrate synthase, isocitrate synthase, pyruvate dehydrogenase, and aconitase genes (paper number 16, page 7, line 23 to page 8, line 2). Consistent with this view previously taken by the Examiner, Applicants have amended the claims to limit the scope of the claimed invention to glutamate dehydrogenase, citrate synthase, isocitrate synthase, pyruvate dehydrogenase, and aconitase genes of coryneform bacteria.

Nonetheless, the Examiner's rejections appear to relate to two aspects of the claims: (1) the genes and (2) the promoters for those genes.

In regard to the first issue, as stated above, Applicants note that the claims have been limited to genes from coryneform bacteria. The Examiner is directed to page 5, line 12 to page 8, line 2 of the specification where bacteria of the genus Corynebacterium, and enzymes (genes) and their corresponding activities are described. Applicants note that due to the high degree of genetic similarity shared by the members of this genus of bacteria, with the present specification in hand, the skilled artisan would be able to readily identify the genes encoding glutamate dehydrogenase, citrate synthase, isocitrate synthase, pyruvate dehydrogenase, and aconitase genes from coryneform bacteria.

Moreover, Applicants note that the actual gene sequence of the genes encoding the aforementioned enzymes is not particularly limiting. As described throughout the specification and the claims, the present invention lies in the creation of a mutant expression sequence where the promoter region of the aforementioned genes is mutated to include the specifically claimed hexamer sequences at the –35 or –10 position of the promoter sequence for these genes.

Applicants remind the Examiner that MPEP §2164.05(a) states:

> "The specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public... The state of the art existing at the filing date of the application is used to determine whether a particular disclosure is enabling as of the filing date."

Therefore, Applicants submit that they are under no obligation to explicitly disclose each and every sequence that falls within the scope of the claimed invention.

In regard to the promoter sequences (point (2) above), Applicants direct the Examiner's attention to page 8, line 3 to page 10, line 22, which fully describe representative promoters of the claimed invention, as well as the creation of a "consensus sequence." Further, as evidence of the fact that the claimed invention is adequately described and enabled for any coryneform bacteria, Applicants **submit herewith** the results of a sequence alignment of the promoter sequences of *Corynebacterium glutamicum* and other members of the Corynebacterium genus.

In the alignment results:

(1) represents the alignment for the *gdh* gene between *Corynebacterium glutamicum* and *Brevibacterium flavum*. –35 and –10 regions are indicated in the box.

(2) represents the alignment for the *gltA* (CS) gene between *Corynebacterium glutamicum* and *Corynebacterium efficiens*. –35 and –10 regions are indicated in the box.

(3) represents the alignment for the *lcd* gene between *Corynebacterium glutamicum* and *Brevibacterium flavum*. –35 and –10 regions are underlined.

(4) represents the alignment for the *pdh* gene between *Corynebacterium glutamicum* and *Brevibacterium flavum*. –35 and –10 regions are underlined.

As evidenced by the enclosed BLASTN search results, the promoter regions of the *gdh*, *gltA*, *icd*, and *pdh* genes are well conserved among coryneform bacteria, although the gdh promoter region of *Brevibacterium flavum*, the gltA promoter region of *Corynebacterium efficiens*, the lcd promoter region of *Brevibacterium flavum*, and the pdh promoter region of *Brevibacterium flavum* possess slight variations from the corresponding regions of *Corynebacterium glutamicum*. Accordingly, based on the present specification supplemented by the enclosed search results, one of skill in the art would be able to immediately identify these promoter regions and mutate the same in accordance with the claimed invention without undue experimentation.

In view of the foregoing, Applicants submit that the claimed invention is adequately described and enabled. Withdrawal of these grounds of rejection is requested.

The objection to the specification as introducing new matter based on the newly submitted substitute Sequence Listing containing 66 sequences rather than the original 64 sequences is traversed.

The Examiner has issued this new matter rejection based on the occurrence of 66 sequences in the substitute Sequence Listing filed November 21, 2003 compared to only 64 sequences in the original Sequence Listing. Applicants wish to direct the Examiner's attention to the fact that original SEQ ID NOs: 31 and 32 contained CD regions and the corresponding protein sequence encoded thereby; however, were not individually listed. Therefore, in order to correct this oversight in the original Sequence Listing and to ensure compliance with the Sequence Rules (37 C.F.R. §1.821-1.825), a substitute Sequence Listing was filed in which the protein sequences were inserted and the remaining sequence identifiers were amended to maintain their corresponding original designation.

In view of the foregoing, Applicants submit that the submission of the Substitute Sequence Listing does not introduce new matter. Withdrawal of this ground of objection is requested.

The objection to the specification as introducing new matter based on the correction of the typographical error in which original SEQ ID NO:62 (now SEQ ID NO:64) contained an "f" at position 29 rather than "g" is traversed.

Applicants again note that the amendment to SEQ ID NO: 64 (previously SEQ ID NO: 62) at position 29 was to correct an inadvertent entry of "f" in place of the appropriate "g". Although the Examiner surely concedes that "f" is not an appropriate nucleic acid designator; however, the Examiner does not believe that there is sufficient evidence to conclude that the proper substitute is "g".

To this end, Applicants refer to page 55, lines 3-6, where they describe SEQ ID NO: 64 (previously SEQ ID NO: 62) as being a primer used for amplification of the *gdh* promoter. Therefore, based on the description and publicly available databases (e.g., GenBank), the proper sequence of the *gdh* promoter region would result in the disclosed primer containing a "g" at this position. To this end, Applicants **submit herewith** the GenBank entry for Accession No. X72855. The inventor used SEQ ID NO: 64 to amplify the promoter region of *gdh* region for constructing the mutated *gdh* promoter. The sequence of SEQ ID NO: 64 is complementary to the sequence ranging from nucleotide 235 to 291 of Accession No. X72855 with flanking mutation and addition of restriction enzyme recognition sites. Based on the complementarity of these sequences it is clear that the position corresponding to nucleotide 29 of SEQ ID NO: 64 is nucleotide 270 in Accession No. X72855, which has a "c" in Accession No. X72855. The antisense nucleotide of "c" is "g" and, therefore, the

skilled artisan would readily appreciate that the correct nucleotide at position 29 of SEQ ID NO: 64 is "g."

In view of the foregoing, Applicants submit that the amendment of SEQ ID NO: 64 (previously SEQ ID NO: 62) does not constitute new matter. Withdrawal of this ground of objection is requested.

The objections to the claims and the rejection of Claims 20-37 under 35 U.S.C. §112, second paragraph, are obviated by amendment.

Applicants have amended the claims to be free of the Examiner's criticisms. Applicants note that the designation of the "–35" and "-10" regions as they relate to promoter sequences are well known the art. Evidence of the ubiquitous knowledge of these terms is offered by the **enclosed** page 860 of Lehninger et al, *Principles of Biochemistry*, 2nd Ed., 1993. As this reference clearly shows, as of the filing date of the present invention, the skilled artisan would readily understand the meaning of these terms.

To this end, Applicants again wish to thank Examiner Prouty for the suggestion to their undersigned Representative to amend the claims to define the promoter sequence as a "DNA sequence situated at about position -35 from the transcription start site of the glutamic acid synthesizing gene" or a "DNA sequence situated at about position -10 from the transcription start site of the glutamic acid synthesizing gene" to clearly convey the scope of the claimed invention in terms that would be readily appreciated by the skilled artisan.

Applicants request withdrawal of these grounds of objection and the indefiniteness rejection.

The rejection of Claims 20-37 under 35 U.S.C. §101 is obviated by amendment.

Consistent with the Examiner suggestion on page 5 of the February 10, 2004 Office Action, Applicants have amended the claims to define the "coryneform bacterium glutamic acid synthesizing gene" as being "isolated." In view thereof, Applicants request that the Examiner acknowledge withdrawal of this ground of rejection.

Finally, in regard to method Claims 38 and 46, Applicants remind the Examiner that MPEP §821.04 states:

> ...if applicant elects claims directed to the product, and a product claim is subsequently found allowable, withdrawn process claims which depend from or otherwise include all the limitations of the allowable product claim *will be rejoined.* (emphasis added)

Accordingly, should the claims corresponding to the elected group (Claims 20-37 and 39-45) be found allowable, the Office is compelled to rejoin non-elected process Claims 38 and 46.

Applicants submit that the present application is now in condition for allowance. Early notification of such action is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Stephen G. Baxter
Attorney of Record
Registration No. 32,884

Vincent K. Shier, Ph.D.
Registration No. 50,552

Customer Number
22850

Tel: (703) 413-3000
Fax: (703) 413-2220
(OSMMN 08/03)
SGB:VKS

BLASTN Search Result

Computed at GenomeNet BLAST2 Server (Kyoto Center) on Fri Apr 16 13:13:12 JST 2004

Database Name NR-NT
>query
CAAACAATTT TAATTCTTTG TGGTCATATC TGTGCGACAC TGCCATAATT
GAACGTGAGC ATTTACCAGC

BLASTN 2.2.6 [Apr-09-2003]

(1)

Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

Query= query
(70 letters)

Database: nr-nt: Non-redundant nucleic acid sequence database Release 04-04-16
33,709,186 sequences; 38,978,163,883 total letters

1. Corynebacterium gdh promoter region.

Searching.....done

Sequences producing significant alignments:

	Score (bits)	E Value
Top 10 Clear Select operation Exec		
☑ emb:AX127150 [AX127150] Sequence 7066 from Patent EP1108790.	139	2e-30
☑ emb:CGGDHA [X72855] C.glutamicum GDHA gene	139	2e-30
☑ emb:AP005280 [AP005280] Corynebacterium glutamicum ATCC 13032 DN...	139	2e-30
☑ gbu:BX927154 [BX927154] Corynebacterium glutamicum ATCC 13032, I...	139	2e-30
☑ emb:CGGDH [X59404] Corynebacterium glutamicum, gdh gen for gluta...	107	8e-21
☑ emb:BD177703 [BD177703] Process for producing L-glutamine by fer...	56	3e-05
☑ emb:AX503514 [AX503514] Sequence 17 from Patent EP1229121.	56	3e-05
☑ emb:BD177706 [BD177706] Process for producing L-glutamine by fer...	50	0.002
☑ emb:AX503517 [AX503517] Sequence 20 from Patent EP1229121.	50	0.002
☑ emb:BD177707 [BD177707] Process for producing L-glutamine by fer...	42	0.40
☐ emb:AX503518 [AX503518] Sequence 21 from Patent EP1229121.	42	0.40
☐ emb:AL806526 [AL806526] Mouse DNA sequence from clone RP23-123F2...	42	0.40
☐ emb:AL159970 [AL159970] Human DNA sequence from clone RP11-140I1...	40	1.6
☐ emb:AP004495 [AP004495] Lotus corniculatus var. japonicus genomi...	40	1.6
☐ emb:AL805970 [AL805970] Mouse DNA sequence from clone RP23-43803...	40	1.6

>emb:AX127150 [AX127150] Sequence 7066 from Patent EP1108790.
Length = 349980

C. glutamicum

Score = 139 bits (70), Expect = 2e-30
Identities = 70/70 (100%)
Strand = Plus / Minus

Query: 1 caaacaattttaattctttgtggtcatatctgtgcgacactgccataattgaacgtgagc 60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

BLAST2 Search Result

```
Sbjct: 96424 caaacaattttaattctttgtggtcatatctgtgcgacactgccataattgaacgtgagc 96365


Query: 61    atttaccagc 70
              ||||||||||
Sbjct: 96364 atttaccagc 96355


>emb:CGGDHA [X72855] C.glutamicum GDHA gene
          Length = 2037

 Score =  139 bits (70), Expect = 2e-30
 Identities = 70/70 (100%)
 Strand = Plus / Plus


Query: 1   caaacaattttaattctttgtggtcatatctgtgcgacactgccataattgaacgtgagc 60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 231 caaacaattttaattctttgtggtcatatctgtgcgacactgccataattgaacgtgagc 290


Query: 61  atttaccagc 70
            ||||||||||
Sbjct: 291 atttaccagc 300


>emb:AP005280 [AP005280] Corynebacterium glutamicum ATCC 13032 DNA,
              complete genome, section 7/10.
          Length = 337200

 Score =  139 bits (70), Expect = 2e-30
 Identities = 70/70 (100%)
 Strand = Plus / Minus


Query: 1       caaacaattttaattctttgtggtcatatctgtgcgacactgccataattgaacgtgagc 60
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 196424 caaacaattttaattctttgtggtcatatctgtgcgacactgccataattgaacgtgagc 196365


Query: 61      atttaccagc 70
                ||||||||||
Sbjct: 196364 atttaccagc 196355


>gbu:BX927154 [BX927154] Corynebacterium glutamicum ATCC 13032, IS
              fingerprint type 4-5, complete genome; segment 7/10.
          Length = 349575

 Score =  139 bits (70), Expect = 2e-30
 Identities = 70/70 (100%)
 Strand = Plus / Minus


Query: 1       caaacaattttaattctttgtggtcatatctgtgcgacactgccataattgaacgtgagc 60
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 71480 caaacaattttaattctttgtggtcatatctgtgcgacactgccataattgaacgtgagc 71421


Query: 61     atttaccagc 70
               ||||||||||
Sbjct: 71420 atttaccagc 71411


>emb:CGGDH [X59404] Corynebacterium glutamicum gdh gen for
```

glutamate dehydrogenase
Length = 2037

Score = 107 bits (54), Expect = 8e-21
Identities = 64/66 (96%), Gaps = 1/66 (1%)
Strand = Plus / Plus

```
Query: 6   aattttaattctttgtggtcatatctgtgcgacactgccataa-ttgaacgtgagcattt 64
           ||||||||||||||||||||||||||||||||||||||||| |||||||||||| ||
Sbjct: 236 aattttaattctttgtggtcatatctgtgcgacactgccataatttgaacgtgagcactt 295

Query: 65  accagc 70
           ||||||
Sbjct: 296 accagc 301
```

Brevibacterium flavum.

>emb:BD177703 [BD177703] Process for producing L-glutamine by fermentation and L-glutamine-producing microorganism.
Length = 44

Score = 56.0 bits (28), Expect = 3e-05
Identities = 40/44 (90%)
Strand = Plus / Plus

```
Query: 16 ctttgtggtcatatctgtgcgacactgccataattgaacgtgag 59
           |||||| | ||||||||||||||| |||| ||||||||||||||
Sbjct: 1   ctttgttgccatatctgtgcgacgctgctataattgaacgtgag 44
```

>emb:AX503514 [AX503514] Sequence 17 from Patent EP1229121.
Length = 44

B. flavum.

Score = 56.0 bits (28), Expect = 3e-05
Identities = 40/44 (90%)
Strand = Plus / Plus

```
Query: 16 ctttgtggtcatatctgtgcgacactgccataattgaacgtgag 59
           |||||| | ||||||||||||||| |||| ||||||||||||||
Sbjct: 1   ctttgttgccatatctgtgcgacgctgctataattgaacgtgag 44
```

>emb:BD177706 [BD177706] Process for producing L-glutamine by fermentation and L-glutamine-producing microorganism.
Length = 29

Brevibacterium flavum

Score = 50.1 bits (25), Expect = 0.002
Identities = 28/29 (96%)
Strand = Plus / Plus

←野性型 Wild type

```
Query: 21 tggtcatatctgtgcgacactgccataat 49
           |||||| |||||||||||| |||||||||
Sbjct: 1  tggtcatatctgtgcgacgctgccataat 29
```

>emb:AX503517 [AX503517] Sequence 20 from Patent EP1229121.
Length = 29

Score = 50.1 bits (25), Expect = 0.002
Identities = 28/29 (96%)
Strand = Plus / Plus

4

BLASTN Search Result

Computed at GenomeNet BLAST2 Server (Kyoto Center) on Fri Apr 16 13:24:39 JST 2004

Database Name NR-NT
>query
aattggctct cacttcggat atggctaaac cgcatttatc ggtatagcgt
gttaaccgga ccagattggg

BLASTN 2.2.6 [Apr-09-2003]

(2)

Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

Query= query
(70 letters)

Database: nr-nt: Non-redundant nucleic acid sequence database Release 04-04-16
33,709,186 sequences; 38,978,163,883 total letters

Searching.....done

Sequences producing significant alignments:

	Score (bits)	E Value
Top 10 Clear Select operation Exec		
emb:AX127145 [AX127145] Sequence 7061 from Patent EP1108790.	139	2e-30
emb:CGGLTG [X66112] C.glutamicum glt gene for citrate synthase a...	139	2e-30
gmb:AP005276 [AP005276] Corynebacterium glutamicum ATCC 13032 DN...	139	2e-30
gbu:BX927150 [BX927150] Corynebacterium glutamicum ATCC 13032, l...	139	2e-30
emb:AP005217 [AP005217] Corynebacterium efficiens YS-314 DNA, co...	52	4e-04
emb:AE015456 [AE015456] Shewanella oneidensis MR-1 section 5 of ...	38	6.2

>emb:AX127145 [AX127145] Sequence 7061 from Patent EP1108790.
Length = 349980

gltA gene.

Score = 139 bits (70), Expect = 2e-30
Identities = 70/70 (100%)
Strand = Plus / Plus

```
Query: 1      aattggctctcacttcggatatggctaaaccgcatttatcggtatagcgtgttaaccgga 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 277660 aattggctctcacttcggatatggctaaaccgcatttatcggtatagcgtgttaaccgga 277719

Query: 61     ccagattggg 70
              ||||||||||
Sbjct: 277720 ccagattggg 277729
```

>emb:CGGLTG [X66112] C.glutamicum glt gene for citrate synthase and ORF
Length = 3013

BLAST2 Search Result

```
Score =  139 bits (70), Expect = 2e-30
Identities = 70/70 (100%)
Strand = Plus / Plus

Query: 1   aattggctctcacttcggatatggctaaaccgcatttatcggtatagcgtgttaaccgga 60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 660 aattggctctcacttcggatatggctaaaccgcatttatcggtatagcgtgttaaccgga 719

Query: 61  ccagattggg 70
           ||||||||||
Sbjct: 720 ccagattggg 729

>emb:AP005276 [AP005276] Corynebacterium glutamicum ATCC 13032 DNA,
          complete genome, section 3/10.
        Length = 332050

 Score =  139 bits (70), Expect = 2e-30
 Identities = 70/70 (100%)
 Strand = Plus / Plus

Query: 1      aattggctctcacttcggatatggctaaaccgcatttatcggtatagcgtgttaaccgga 60
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 209710 aattggctctcacttcggatatggctaaaccgcatttatcggtatagcgtgttaaccgga 209769

Query: 61     ccagattggg 70
          ||||||||||
Sbjct: 209770 ccagattggg 209779

>gbu:BX927150 [BX927150] Corynebacterium glutamicum ATCC 13032, IS
          fingerprint type 4-5, complete genome; segment 3/10.
        Length = 348475

 Score =  139 bits (70), Expect = 2e-30
 Identities = 70/70 (100%)
 Strand = Plus / Plus

Query: 1      aattggctctcacttcggatatggctaaaccgcatttatcggtatagcgtgttaaccgga 60
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 181273 aattggctctcacttcggatatggctaaaccgcatttatcggtatagcgtgttaaccgga 181332

Query: 61     ccagattggg 70
          ||||||||||
Sbjct: 181333 ccagattggg 181342

>emb:AP005217 [AP005217] Corynebacterium efficiens YS-314 DNA, complete
          genome, section 4/11.
        Length = 300750

 Score = 52.0 bits (26), Expect = 4e-04
 Identities = 41/46 (89%)
 Strand = Plus / Plus

Query: 17    ggatatggctaaaccgcatttatcggtatagcgtgttaaccggacc 62
          ||||||||||| ||| | ||||||||||||||| ||||||
Sbjct: 22597 ggatatggctaaacggcaatcgtcggtatagcgtgttaaacggacc 22642
```

Corynebacterium efficiens.

PICD (3)

```
2256 : 1   caacccacgttcaggtgactactactggattgctggcgaaagtggtgtcgtgaccagcat 60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
2247 : 280 caacccacgttcaggtgactactactggattgctggcgaaagtggtgtcgtgaccagcat 221

2256 : 61  tcgtcgatctctagtgaaagagaaaggcctcgaccgttc-ccaagtggcattcatggggt 119
           |||||||||||||||||||||||||||||||||||| | ||||||||||||||||||||
2247 : 220 tcgtcgatctctagtgaaagagaaaggcctcgaccgtncnccaagtggcattcatgggct 161

2256 : 120 attggaaacacggcgtttccatgcgggctgaaactgccaccataggcgccagcaattag 179
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
2247 : 160 attggaaacacggcgtttccatgcgggctgaaactgccaccataggcgccagcaattag 101

2256 : 180 t 180
           |
2247 : 100 t 100
```

2247 : Brevibacterium flavum

pPDH (4)

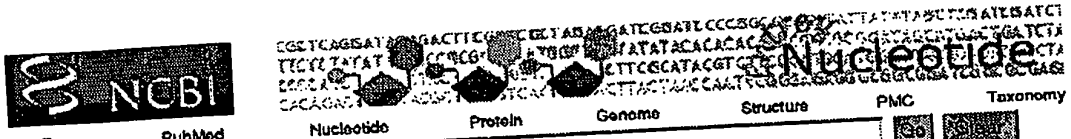
```
2256 : 4   cactccccttacgtcacagtctgtaaaacaaatcttcggtgttgcgtatccttgttaata 63
           ||||  ||  ||||  ||||||||||||||    |  |||||||||||||||||||||||
2247 : 324 cactnccnttacntcacagtctgtaaannnnntnnncggtgttgcgtatccttgttaata 265

2256 : 64  acttatgcgttgacccattcgtgcacttcggtgtgccacaattaggtacgaccaagaatg 123
           ||||||||||||| |||||||||||||||| ||| |||||||||||||||||||||||
2247 : 264 acttatgcgttgactcattcgtgcacttcggcgtgtcacaattaggtacgaccaagaatg 205

2256 : 124 ggaccgggaaaccgggacgtataaacgaaataaaacattccaacaggaggtgtggaaatg 183
           |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
2247 : 204 ggaccgggaaaccgggacgtataaacgaaataaaacattccaacannaggtgtggaaatg 145

2256 : 184 gccgatcaagcaaaact 200
           ||||||||||||||||
2247 : 144 gccgatcaagcaaaact 128
```

2247 : Brevibacterium flavum



1: X72855. C.glutamicum GDHA...[gi:288412]

LOCUS CGGDHA 2037 bp DNA linear BCT 24-MAY-1993
DEFINITION C.glutamicum GDHA gene.
ACCESSION X72855
VERSION X72855.1 GI:288412
KEYWORDS glutamate dehydrogenase.
SOURCE Corynebacterium glutamicum
ORGANISM Corynebacterium glutamicum
Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales;
Corynebacterineae; Corynebacteriaceae; Corynebacterium.

REFERENCE 1 (bases 1 to 2037)
AUTHORS Guyonvarch,A., David,F. and Leblon,G.
TITLE Glutamate dehydrogenase (gdhA) gene
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 2037)
AUTHORS Guyonvarch,A.
TITLE Direct Submission
JOURNAL Submitted (23-MAR-1993) A. Guyonvarch, Institut de Genetique et
Microbiologie, Lab de Biolog Moleo des Corynebacteries, URA D1354
CNRS et GDR 961, Universite Paris-sud, 91405 Orsay Cedex, FRANCE

FEATURES Location/Qualifiers
source 1..2037
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//
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RNA Synthesis Is Initiated at Promoters

Initiation of RNA synthesis at random points in a DNA molecule would be an extraordinarily wasteful process. Instead, the RNA polymerase binds to specific sequences in the DNA called **promoters,** which direct the transcription of adjacent segments of DNA (genes). The sequences adjacent to genes where RNA polymerases must bind can be quite variable, and much research has focused on identifying the sequences that are critical to promoter function. Analysis and comparison of sequences in many different bacterial promoters have revealed similarities in two short sequences located about 10 and 35 base pairs away from the point where RNA synthesis is initiated (Fig. 25–5). By convention the base pair that begins an RNA molecule is given the number +1, so these sequences are commonly called the −10 and −35 regions. The sequences are not identical for all bacterial promoters, but certain nucleotides are found much more often than others at each position. The most common nucleotides form what is called a **consensus sequence** (recall the consensus sequences of *ori*C in the *E. coli* chromosome; see Fig. 24–10). For most promoters in *E. coli* and related bacteria, the consensus sequence for the −10 region (also called the Pribnow box) is (5′)TATAAT(3′), and the consensus sequence at the −35 region is (5′)TTGACA(3′).

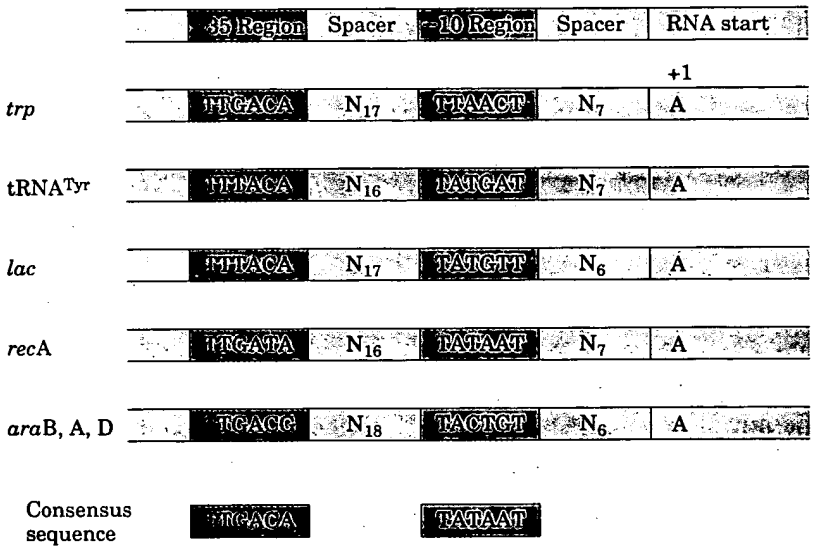

Figure 25–5 The sequences of five *E. coli* promoters. These include promoters for genes involved in *tryp*tophan, *lac*tose, and *ara*binose metabolism. The sequences vary from one promoter to the next, but comparisons of many promoters reveal similarities in the −10 and −35 regions. The consensus sequences of the −10 and −35 regions are shown at the bottom. The −10 region is often called the Pribnow box, after David Pribnow, the investigator who first recognized it in 1975. All sequences shown are those of the coding (nontemplate) strand and read 5′→3′, left to right, as is the convention in representations of this kind. The spacer regions contain variable numbers of nucleotides (N). Only the first nucleotide coding the RNA transcript (at position +1) is shown.

Many independent lines of evidence attest to the functional importance of these sequences. Mutations that affect the function of a given promoter usually involve one of the base pairs in the −35 or −10 region. Natural variations in the consensus sequence also affect the efficiency of RNA polymerase binding and transcription initiation. Differences of a few base pairs can decrease the rate of initiation by several orders of magnitude, providing one means by which *E. coli* can modulate the expression of different genes. In addition, specific binding of RNA polymerase to these sequences has been directly demonstrated in vitro (Box 25–1).

SECOND EDITION

Principles of Biochemistry

with an Extended Discussion of Oxygen-Binding Proteins

Albert L. Lehninger

Late University Professor of Medical Sciences
The Johns Hopkins University

David L. Nelson

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WORTH PUBLISHERS

Principles of Biochemistry Second Edition

Albert L. Lehninger, David L. Nelson, and Michael M. Cox

Printed in the United States of America

Library of Congress Catalog Card No. 94-60553

ISBN: 0-87901-500-4

ISBN: 0-87901-711-2 with supplement

Printing: 5 4 3 Year: 97 96 95 94

Development Editor: Valerie Neal

Design: Malcolm Grear Designers

Art Director: George Touloumes

Project Editor: Elizabeth Geller

Production Supervisor: Sarah Segal

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Picture Editor: Stuart Kenter

Illustration Design: Susan Tilberry

Illustrators: Susan Tilberry, Alan Landau, and Joan Waites

Computer Art: Laura Pardi Duprey and York Graphic Services

Composition: York Graphic Services

Printing and binding: R.R. Donnelley and Sons

Cover: The active site of the proteolytic enzyme chymotrypsin, showing the substrate (blue and purple) and the amino acid residues (red and orange) critical to catalysis. Determination of the detailed reaction mechanism of this enzyme (described on pp. 223–226) helped to establish the general principles of enzyme action.

Frontispiece: A view of tobacco ribulose-1,5-bisphosphate carboxylase (rubisco). This enzyme is central to photosynthetic carbon dioxide fixation; it is the most abundant enzyme in the biosphere. Different subunits are shown in blues and grays. Important active site residues are shown in red. Sulfates bound at the active site (an artifact of the crystallization procedure) are shown in yellow.

Cover, frontispiece, and part opening images produced by Alisa Zapp (see *Molecular Modeling* credits, p. IC-4) and enhanced by Academy Arts.

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Worth Publishers

33 Irving Place

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